Presence of Macrophage-tropic variants following Analytical Treatment Interruption

Viviane Machado Andrade[1], Carla Mavian[2], Dunja Babic[1], Thaissa Alvarado[1], Mark Sharkey[1], Labelle Barrios[1], Christian Brander[3], Javier Martinez-Picado[3], Jonathan Li[4], Michael Seaman[5], Christy Lavine[5], Marco Salemi[2], Mario Stevenson[1]

[1]Department of Medicine, University of Miami. [2]Emerging Pathogens Institute. University of Florida. [3]IrsiCaixa AIDS Research Institute, Catalonia, Spain. [4]Brigham and Women's Hospital, Harvard Medical School. [5]Beth Israel Deaconess Medical Center, Harvard Medical School

Introduction

HIV persists in cell reservoirs that are not eliminated by cART.

Macrophages may be contributing to HIV persistence:

- Macrophages survive from HIV infection and become long-lived.
- HIV infection is compartmentalized in the brain.
- Macrophages reside in tissues under sub-optimal antiretroviral drug concentration.

Challenges:

- Macrophages reside in nearly all tissues (including the CNS), and sample biopsies are not easily accessible.
- Tissue biopsies represent a small portion of the whole tissue, which can be a biased representation of the infection.
- Purification of macrophages without CD4+ T cell contamination is laborious.

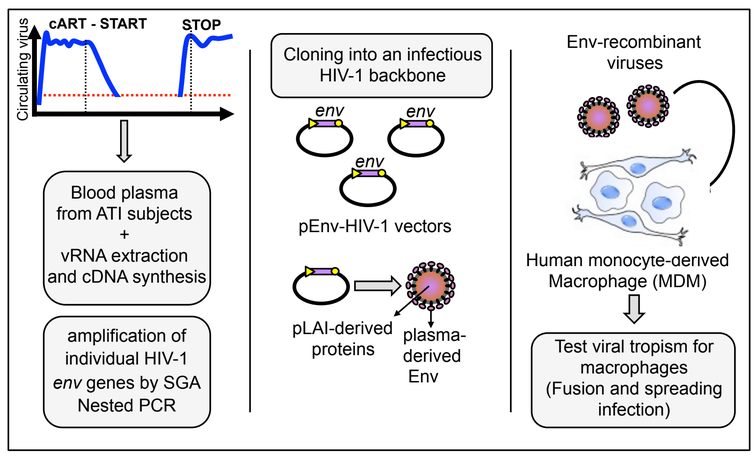
Rationale:

- When ART is interrupted, HIV-1 rebounds from cellular reservoirs.
- By tracking rebound viruses, we may be able to find the reservoir.
- Tropism to viral reservoirs is given by interaction between the HIV-1 envelope and the cellular receptors of a target cell (CD4 and co-receptors).
- Macrophage-tropic virus: viruses that have adapted to use low levels of CD4 receptor to infect macrophages.

Hypothesis: macrophages are a reservoir for HIV-1 persistence in individuals on ART.

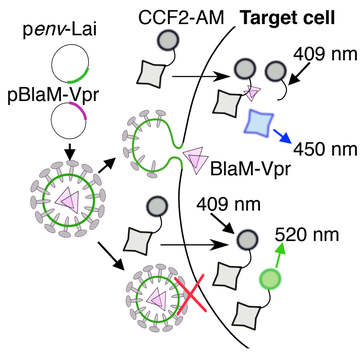
Research Strategy

a)



Fusion-mediated entry of control viruses in infected macrophages

b)



c)

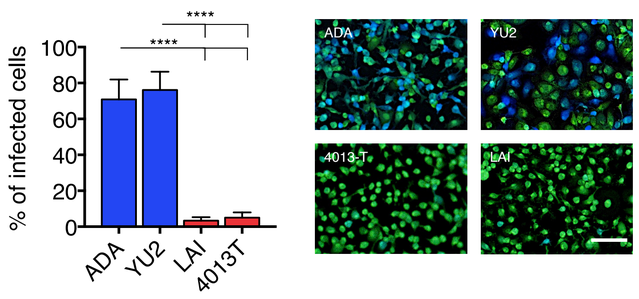


Figure 1. (a) Schematic representation of the strategy for generation of HIV-1 envelopes from plasma and construction of recombinant HIV-1 clones harboring patient-derived envelopes. (b) Assessment of HIV-1 fusogenicity for primary macrophages. The fusion assay consists in the cleavage of CCF2 by an encapsidated β-lactamase. Hydrolyzed (indicative of fusion) and non-hydrolyzed CCF2-AM are distinguished by examination of infected cells at 450 nm. (c) Fusogenic capacities of macrophage and T-cell adapted laboratory controls on primary macrophages. Highly macrophage-tropic controls (YU2, ADA) are displayed as blue bars and T-tropic controls (LAI, 4013P) in red. Bars are representative of at least 47 independent macrophage donors. Error is displayed as mean ± s.d. ****P <0.0001 (two-tailed unpaired t-test). Images are representative fields of each of the controls.

Results

Study participant characteristics

PID	Age	Time on ART	ART regimen prior to ATI	Plasma collection (days post-ATI)	ATI viral load (copies/mL)	CD4+ T cells / ml prior to ATI
63	49	4.2	3TC, AZT, NFV	29	750,000	1292
102	42	2.5	3TC, NFV, NVP	27	750,000	990
109	37	3.7	D4T, DDI, NVP	28	164,000	1312
126G	41	11.9	EFV, CMBV, DDI	29	1,054,109	836
216	N/A	N/A	N/A	26	415,000	N/A
234/1	41	8.7	D4T, IDV, 3TC	48	354,000	373
PL234/2	41	8.7	D4T, IDV, 3TC	57	270,000	373

N/A: not available.

Macrophage-tropic viruses are found in rebound plasma viremia

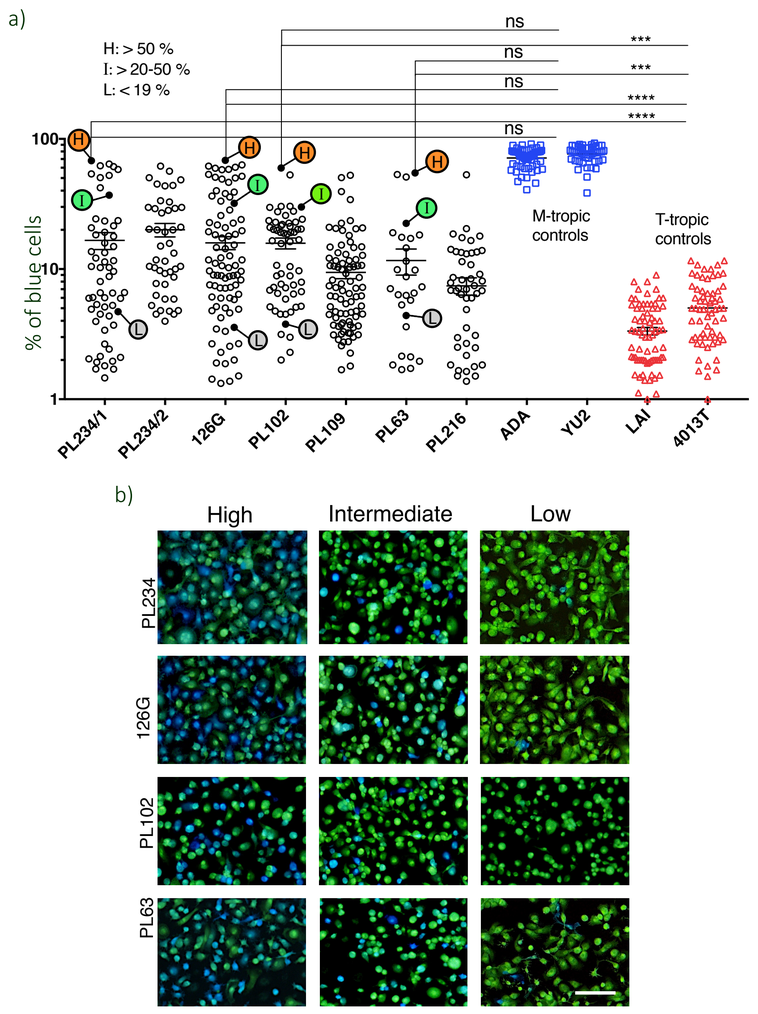
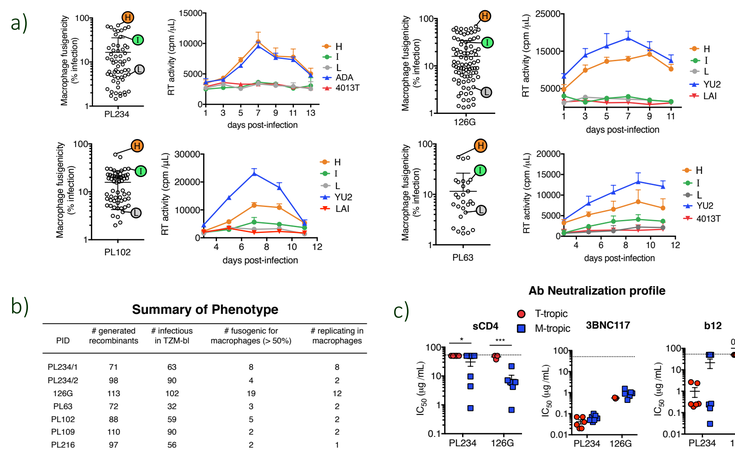


Figure 2. (a) Fusogenicities of post-ATI-derived patient envelopes for macrophages. Fusion capacity in macrophages was assessed for approximately 500 recombinant viruses from six subjects undergoing ATI. Representative recombinants exhibiting high (H, greater than 50% macrophages with hydrolyzed CCF2-AM), intermediate (I, 20-50%) and low (L, <20%) macrophage fusogenicity are indicated for each subject. (b) Representative microscopic images for viruses designated H, I and L are shown. Each data set represents the mean of 3 independent macrophage preparations from independent donors. Graph is shown as mean ± s.e.m. ***P < 0.001, ****P < 0.0001 (two-tailed unpaired t-test). Scale bar = 100 µm.

Macrophage-tropic viruses replicate in macrophages and are sensitive to soluble CD4



PID	# generated recombinants	# infectious in TZM-bl	# fusogenic for macrophages (> 50%)	# replicating in macrophages
PL234/1	71	63	8	8
PL234/2	98	90	4	2
126G	113	102	19	12
PL63	72	32	3	2
PL102	88	59	5	2
PL109	110	90	2	2
PL216	97	56	2	1

Figure 3. Selected recombinant viruses with high, intermediate, and low capacity for fusing with macrophages were assessed for their ability to undergo spreading infection in these cells. Replication was assessed by HIV-1 reverse transcriptase activity in the culture supernatants. Graphs are displayed as mean ± s.d. (b) Summary of viral infectivity phenotypes assessed by fusion and replication capacity in TZM-bl and macrophages. (c) Antibody and CD4 inhibitor sensitivity of recombinant viruses harboring PL234 and 126G patient derived envelopes with high (M-tropic) and low (T-tropic) macrophage fusogenicities. Dashed line indicates highest antibody concentration tested. Error bars are shown as mean ± s.em. *P < 0.05, ***P < 0.001 (two-tailed unpaired t-test).

Macrophage-adapted variants have a macrophage origin

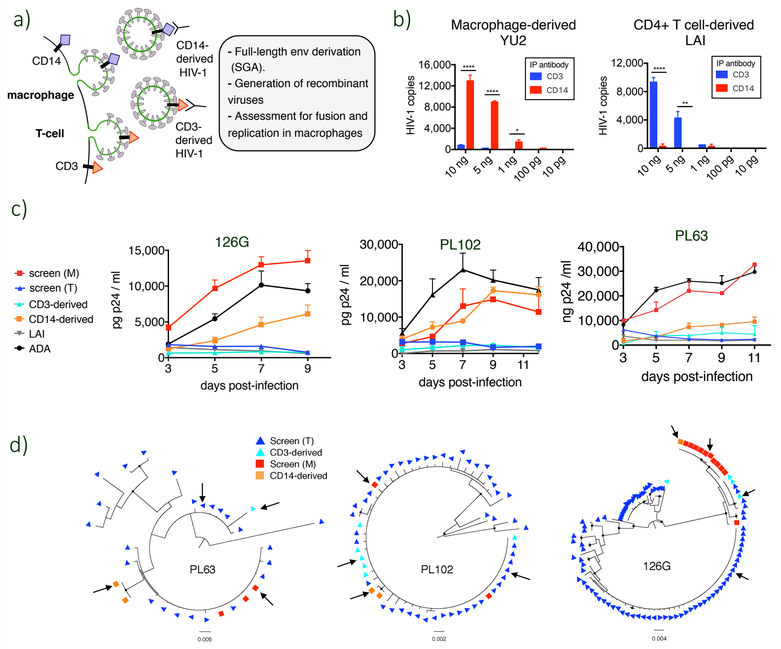


Figure 4. (a) Rationale for antibody-mediated enrichment of macrophage-adapted virions from post-ATI plasma. Virions in post-ATI plasma were immunoprecipitated with CD14 or CD3 antibodies. (b) Levels of enrichment using CD3 and CD14 control antibodies were assessed on virions derived in vitro from macrophages and CD4+ T cells. Total HIV-1 DNA was quantified from immunoprecipitates by RT-PCR. Error bars are representative of two independent experiments. ****P < 0.0001, *P < 0.05 (two-way ANOVA, multiple comparison). (c) Replication capacity of recombinant viruses containing CD14 and CD3-derived envelopes was assessed in parallel with patient-matched T- and M-tropic recombinant viruses whose envelopes had been derived by direct cloning from raw, post-ATI plasma. Error bars are representative of two (126G, PL63) or three (PL102) independent donor cells. Graphs are displayed as mean ± s.e.m. (c) Maximum likelihood phylogenies of HIV-1 env sequences from PL102, PL63 and 126G patients sampled from post-ATI plasma. Arrows indicate representative infectious clones used to evaluate replication in macrophages in (b).

Macrophage-adapted envelopes predated treatment interruption

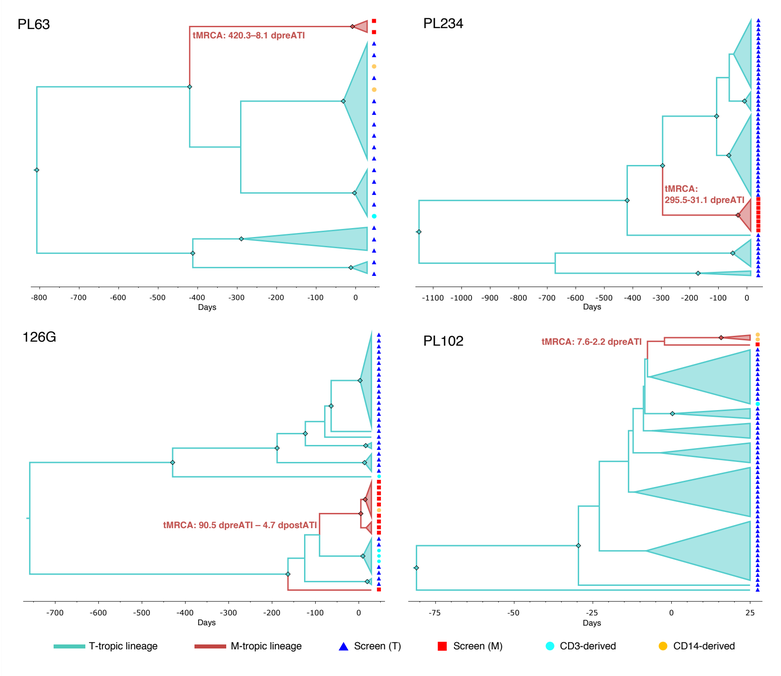


Figure 5. Maximum clade credibility trees inferred from HIV-1 env sequences for each patient were scaled in time by enforcing a molecular clock with prior mean evolutionary rate of 7.53 10^{-3} nt substitutions/site/year. Time scale is in days: 0 corresponds to the time of treatment interruption, days before ART interruption (preATI), and days post ART interruption (postATI). Branches and internal nodes are colored according to phenotype (M-tropic brown, T-tropic cyan); Diamonds represent branches supported by posterior probability >0.99. For simplicity, highly supported clades of T-tropic sequences were collapsed.

References

- Macrophage-tropic envelopes are found in rebound viremia of subjects who underwent treatment interruption.
- Macrophage-adapted variants found in post-ATI viremia confer high fusogenicity and replication capacity in macrophages.
- Macrophage-tropic envelopes are enriched for a macrophage-specific antibody, thus suggesting a macrophage origin.
- Macrophage-tropic envelopes predated treatment interruption, indicating that macrophage-adapted variants persisted under therapy

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